with the synthetical isobutyleneglycol of Nevolé,* which boils at 176° to 178°. The yield obtained from sugar was, however, very small, and only amounted to about 0·2 per cent. after allowing for the losses involved in the extraction of the compound.

In view of the properties of the crude glycol described above, it would seem advisable to re-examine Henninger's glycol, the constitution of which was not experimentally examined.

The Alcoholic Ferment of Yeast-Juice.

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(From the Chemical Laboratory, Lister Institute.)

1. *Effect of the addition of Boiled and Filtered Yeast-juice on the Fermentation of Glucose Produced by Yeast-juice.*

In the course of some experiments on the action of various proteids on the fermentative activity of yeast-juice, it was observed that the alcoholic fermentation of glucose by yeast-juice is greatly increased by the addition of yeast-juice which has been boiled and filtered, either when fresh or after having undergone autolysis, although this boiled liquid is itself incapable of setting up fermentation. Thus, the total fermentation produced by yeast-juice acting on excess of glucose is, as a rule, doubled by the addition of an equal volume of the boiled juice, and a further increase is produced when a greater volume is added, the sugar concentration being kept constant.†

A similar observation was previously made by Buchner and Rapp‡ in a single experiment (No. 265).

The following table embodies a few of the results obtained, the yeast-juice being prepared and the amount of carbon dioxide evolved being estimated by the method previously employed by the authors.* In every case the concentration of sugar was kept constant, and both in these and all the fermentation experiments described in this paper, toluene was added as an antiseptic.

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* 'Compt. Rend.,' 1876, vol. 83, p. 65.

† Harden and Young, Preliminary Note, 'Proc. Physiol. Soc.,' 1904, vol. 32, November 12.

‡ 'Ber.,' 1899, vol. 32, p. 2093.

Table I.—Effect of the Addition of Boiled Yeast-juice on the Total Fermentation of Glucose by Yeast-juice.

No.	Juice.	Water.	Boiled juice.	Glucose.	Time.	Carbon dioxide.
	c.c.	c.c.	c.c.	grammes.	hours.	gramme.
1	25	25	0	5	72	0·137
	25	0	25	5	72	0·378
2	20	20	0	4	44	0·115
	20	0	20	4	44	0·363
3	25	0	0	2·5	40	0·370
	25	0	25	5	40	0·620
4	20	40	0	6	42	0·458
	20	0	40	6	42	0·858
5	25	25	0	5	44	0·346
	25	0	25	5	44	0·709
6	25	25	0	5	48	0·110
	25	0	25	5	48	0·216
7	25	25	0	5	60	0·273
	25	0	25	5	60	0·466
8	25	25	0	5	120	0·424
	25	0	25	5	120	0·959
9	25	25	0	5	72	0·414
	25	20	5	5	72	0·546
	25	10	15	5	72	0·735
	25	5	20	5	72	0·810
	25	0	25	5	72	0·924
10	25	25	0	5	70	0·246
	25	0	25	5	70	0·356
	25	50	0	7·5	70	0·180
	25	0	50	7·5	70	0·431
	25	75	0	10	70	0·141
	25	0	75	10	70	0·515

In Experiments 1 to 5 the juice added had been autolysed before being boiled; in Nos. 6 to 8 the added juice was boiled as soon as it had been prepared. Experiments 9 and 10 show that each successive addition of boiled juice, from 0·2 to 3 volumes, produces a further increase in the amount of the fermentation.

A similar effect is produced, (1) By the precipitate produced in boiled yeast-juice by the addition of 3 volumes of alcohol (Experiment 1, Table II); (2) By the liquid formed by the autoplasmolysis of yeast, when it is allowed to stand at the air temperature for some time (Experiments 2 and 3, Table II); (3) By the liquid obtained by boiling Buchner's "Aceton-Dauerhefe" with water (Experiment 4, Table II). Further, yeast killed by acetone and ether (Aceton-Dauerhefe) reacts with boiled juice in the same way as does yeast-juice (Experiment 5, Table II).

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* Harden and Young, 'Ber.,' 1904, vol. 37, p. 1052.

Table II.—Effect of Various Substances in Increasing Alcoholic Fermentation.

No.	Yeast-juice.	Addition.	Glucose.	Time.	Carbon dioxide.
	c.c.			hours.	gramme.
1	25	25 c.c. water	5	48	0·110
	25	25 c.c. water + precipitate by 75 per cent. alcohol from 25 c.c. boiled fresh juice	5	48	0·268
	25	Filtrate from 25 c.c. boiled fresh juice + 3 volumes alcohol, made to 25 c.c.	5	48	0·141
	25	25 c.c. water + precipitate from 25 c.c. boiled old juice by 75 per cent. alcohol	5	48	0·286
2	25	25 c.c. water	5	72	0·070
	25	25 c.c. autoplasmolysed yeast-juice, made neutral	5	72	0·189
3	25	25 c.c. water	5	72	0·084
	25	25 c.c. autoplasmolysed yeast-juice, made neutral	5	72	0·172
	25	25 c.c. water	5	72	0·475
4	25	25 c.c. aqueous infusion of 2 grammes Aceton-Dauerhefe	5	72	0·625
5	2 grammes Aceton-Dauerhefe	40 c.c. water	4	48	0·062
	”	20 c.c. water + 20 c.c. boiled juice	4	48	0·136

2. *Dialysis of the Boiled Juice.*

The constituent of the boiled and filtered juice to which this effect is due is removed when the liquid is dialysed in a parchment tube, leaving an inactive residue. In the experiments detailed in the following table (Table III, Experiments 1, 2 and 3) the effect of the addition of boiled juice is compared with that produced by the residue and dialysate respectively.

In Experiment 4, the unboiled juice was dialysed, and the fact that the dialysate had a similar effect to a boiled juice shows that the active constituent exists in the original yeast-juice and is not formed during the boiling.

Table III.—Dialysis of Boiled Yeast-juice. 25 c.c. yeast-juice + 5 grammes glucose + toluene.

No.	Water.	Boiled juice.	Residue.	Dialysate.	Time.	Carbon dioxide.
	c.c.	c.c.	c.c.	c.c.	hours.	gramme.
1	25	0	0	0	48	0·253
	0	25	0	0	48	0·561
	0	0	25	0	48	0·264
2	25	0	0	0	48	0·268
	0	25	0	0	48	0·497
	0	0	25	0	48	0·276
3	25	0	0	0	72	0·113
	0	25	0	0	72	0·334
	0	0	25	0	72	0·189
	0	0	0	25	72	0·334
4	25	0	0	0	48	0·154
	0	0	0	25	48	0·251

3. *Dialysis of Yeast-juice.*

The facts above detailed suggested the possibility of dividing yeast-juice into two fractions by dialysis; an inactive residue and a dialysate which, although itself inert, would be capable of rendering this residue active.

This was experimentally realised by filtering the juice through a Martin gelatin filter.*

This method of rapid dialysis was chosen because the yeast-juices at our disposal lost their activity too rapidly to permit of the ordinary process of dialysis through parchment being carried out. Either a 10- or a 7·5-per-cent. solution of gelatin was used to impregnate the Chamberland filter and the filtration was carried out under a pressure of 50 atmospheres.

Only a portion of the juice placed in the filter was actually filtered, the remainder being simply poured out of the case as soon as a sufficient quantity of filtrate had passed through. The residue adhering to the candle, which consisted of a brown viscid mass, was dissolved in water and made up to the volume of the juice filtered. Glucose was then added and one portion incubated at 25° with an equal volume of sugar solution and a second portion with an equal volume of the filtrate or of a boiled juice, containing an equal amount of glucose. Before incubation the carbon dioxide was pumped out of all the solutions. The filtrate was invariably found to be quite devoid of fermenting power, none of the enzyme having passed through the gelatin.

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* 'Journ. Physiol.,' 1896, vol. 20, p. 364.

The results (Table IV) show that in this way an almost inactive residue can be obtained which is rendered active by the addition of the filtrate (Experiments 1, 2, 3) or a boiled juice (Experiment 4).

Table IV.—Filtration of Yeast-juice through the Martin Gelatin Filter.
15 c.c. residue + 3 grammes glucose + toluene.

No.	Water.	Filtrate.	Boiled juice.	Time.	Carbon dioxide.
	c.c.	c.c.	c.c.	hours.	gramme.
1	15	0	0	48	0·000
	0	15	0	48	0·035
2	15	0	0	60	0·001
	0	15	0	60	0·051
3	15	0	0	60	0·008
	0	15	0	60	0·064
4	15	0	0	60	0·024
	0	0	15	60	0·282

The total fermentations observed even in the presence of the filtrate are very low, this being, at all events in part, due to the fact that in this series of experiments the original juices themselves happened to be of low fermenting power.

In a second set of experiments (Table V) a smaller quantity of juice was placed in the filter and the filtration was continued until no more liquid would pass through. The residue was then washed several times by adding water and forcing it through the filter. The time occupied in this process varied greatly with different juices, the limits for the filtration and washing of 50 c.c. of juice, using two filters simultaneously, were about 6 to 12 hours. The carbon dioxide was not estimated by absorption in potash as in the previous cases, but was collected and measured over mercury, by means of the apparatus described later on, the object of this procedure being to ascertain not only the total amount of carbon dioxide produced, but the rate and duration of the evolution. The residue was dissolved in water and made to the same volume as the original juice, and the filtrate was evaporated down to the same volume. All the solutions were saturated with carbon dioxide at the temperature of the bath (25°) before the measurements were commenced, and the observations were continued until all fermentation had ceased.

The boiled juice added in Experiments 1, 3 and 4 (Table V) was obtained by boiling a portion of the same preparation as was used for the filtration. The carbon dioxide is expressed in cubic centimetres under atmospheric conditions.

Table V.—Filtration of Yeast-juice through the Martin Gelatin Filter.

No.	Vol. of juice filtered.	Wash water.	Residue.	Filtrate.	Boiled juice.	Glucose.	Carbon dioxide.
	c.c.	c.c.	c.c.	c.c.	c.c.	grammes.	c.c.
1	75	200	25	0	0	2·5	10·4
			25	0	25	5	396·3
2	80	260	20	0	0	2	8·3
			20	20	0	4	90·2
3	100	250	25	0	0	2·5	0·4
			25	0	25	5	268
4	50	200	25	0	0	2·5	0·9
			25	0	25	5	192

The process of filtration does not always produce an inactive residue, as on several occasions the residue after very thorough washing has been found to retain a considerable amount of activity. No reason has yet been found for this and it has not yet been ascertained whether it is due to some peculiarity in the particular specimen of juice or in the special filter employed.

It is of interest to note that in Experiment 2 (Table V) the residue alone gave 8·3 c.c. of carbon dioxide in 3 hours, the amount evolved in the last hour being only 0·1 c.c. At the close of this period the liquid still contained the alcoholic enzyme, since on the addition of 20 c.c. of the filtrate, fermentation recommenced and continued for many hours.

These two sets of experiments (Tables IV and V) show that the fermentation of glucose by yeast-juice is dependent upon the presence of a dialysable substance which is not destroyed by heat.

4. *Analysis of the Effect of the Addition of Boiled Juice upon the Fermentation of Glucose by Yeast-juice.*

In order to compare the course of the fermentation in the presence and in the absence of boiled yeast-juice, experiments were carried out in which the rate of evolution of carbon dioxide was observed in each case throughout the whole period of activity of the juice, which, as a rule, in presence of an excess of sugar, lasts for about 48 to 60 hours.

For this purpose the fermentation was allowed to proceed in a 100 c.c. flask, kept at the constant temperature of 25° by immersion in a thermostat, and connected with an azotometer, in which the gas was collected over mercury. The gas in the fermentation flask was maintained at a constant pressure, as nearly as possible that of the atmosphere, by keeping the mercury in the reservoir at a fixed level, by means of a syphon dipping into a small beaker. The volume of the gas was read on the azotometer without disturbing the mercury reservoir and was reduced to atmospheric pressure by means of a calibration curve. Since yeast-juice readily becomes supersaturated with carbon dioxide, the contents of the flask were vigorously shaken before each reading of the volume of gas. Before the observations were commenced the liquids were brought to the temperature of the thermostat, and were saturated with carbon dioxide. In all comparative experiments the concentration of glucose was the same.

When the rates of evolution of carbon dioxide from (1) a solution of glucose in yeast-juice, and (2) a similar solution to which boiled and filtered yeast-juice has been added are compared, it is found that two phenomena are concerned in the production of the increased fermentation in the presence of boiled yeast-juice.

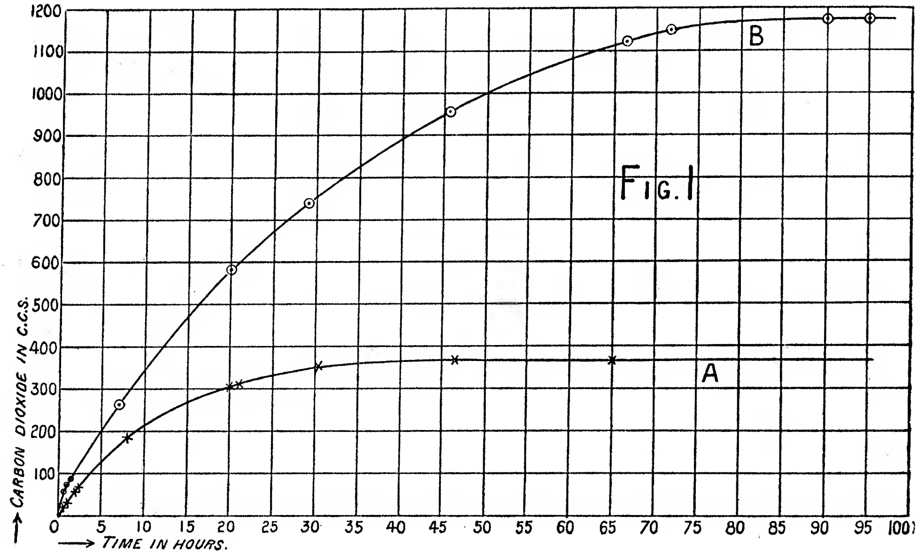
(*a*) An initial rapid evolution of carbon dioxide is produced, which soon diminishes until a rate is attained which remains nearly constant for several hours and is usually, but not invariably, approximately equal to that given by an equal volume of the same yeast-juice and glucose to which no addition has been made.

(*b*) The fermentation rate diminishes more slowly, so that the fermentation continues for a longer period. The greater proportion of the total increase is usually due to this second phenomenon.

The results obtained in a typical experiment of this kind are shown in Fig. 1. The initial period of the evolution is plotted separately (Curves A′ and B′) on a larger scale.

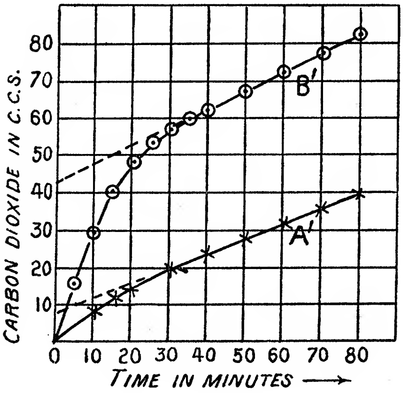
Curves A and A′ in which the evolution of carbon dioxide is plotted against time represent the course of a fermentation with 25 c.c. yeast-juice + 25 c.c. water + 5 grammes glucose + toluene. The rate to begin with is 48 c.c. per hour, but rapidly decreases until it becomes equal to 24 c.c. per hour, at which it remains almost constant for about 5 hours, gradually decreasing until, after the expiration of about 40 to 45 hours, fermentation ceases. The total evolution amounted to 369 c.c. under atmospheric conditions.

Curves B and B′ refer to 25 c.c. of the same yeast-juice + 25 c.c. of a boiled yeast-juice + 5 grammes glucose + toluene. The initial rate is much higher, 168 c.c. per hour, but this falls gradually in the course of 40 minutes to 30 c.c. per hour. This rate of 30 c.c. per hour falls off much less rapidly than that in Experiment A, the fermentation continuing for about 80 to 85 hours and yielding in all 1174 c.c. of carbon dioxide. It is important to bear in mind that these curves represent the gradual disappearance of the fermenting power



of the liquid, and not the diminution of the amount of fermentation with diminishing concentration of sugar, an excess of this substance being present throughout.

A comparison of the two curves shows very clearly the two factors involved in the great increase in Experiment B: (1) The initial rapid evolution, and (2) the prolongation of the fermentation.



5. *The Initial Period of Rapid Evolution of Carbon Dioxide.*

This is a very striking phenomenon, and a typical example is illustrated in fig. 2 in which the curves show the course of the evolution of carbon dioxide (total volume evolved plotted against time) during two hours in the case of:

A. 25 c.c. yeast-juice + 75 c.c. water + 10 grammes glucose + toluene.

B. 25 c.c. yeast-juice + 50 c.c. water + 25 c.c. boiled autolysed yeast-juice + 10 grammes glucose + toluene.

C. 25 c.c. yeast-juice + 75 c.c. boiled autolysed yeast-juice + 10 grammes glucose + toluene.

In B and C the initial rates are almost equal (58 c.c. in 10 minutes) and much greater than in A (14 c.c. in 10 minutes). In B the rate rapidly falls off whilst in C it diminishes much more slowly. A similar initial period is also observable in A, but is not nearly so marked.

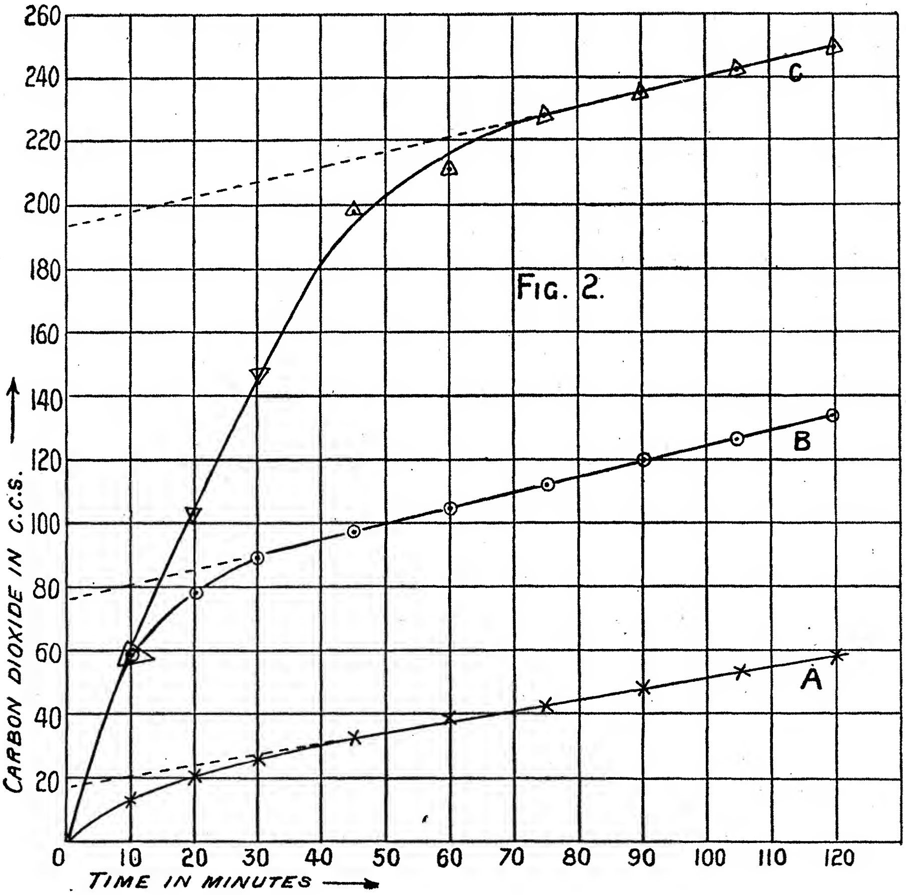


Fig. 2.

The extra quantity of carbon dioxide evolved in this initial period may be calculated by subtracting the amount corresponding with the constant rate which is finally attained from the total amount observed. This is done graphically in fig. 2 by continuing the straight line representing the constant rate back to the axis of ordinates. The following numbers are thus obtained: for A, 16·6; for B, 75·4; for C, 192·9.

The amounts due to the addition of boiled juice are therefore: for 1 volume in B, 75·4 − 16·6 = 58·8; for 3 volumes in C, 192·9 − 16·6 = 176·3 = 3 × 58·8.

The extra amount of carbon dioxide is, therefore, directly proportional to the volume of boiled juice added.

6. *Production of the Initial Rapid Evolution of Carbon Dioxide by the Addition of Phosphates.*

As the result of a large number of attempts to isolate the constituent of boiled juice which brings about the increase in fermentation, it was found that whenever an increase was produced phosphoric acid in the form of a soluble phosphate was present. The effect of the addition of soluble phosphates to yeast-juice was, therefore, examined and it was found that a well-marked initial rapid evolution of carbon dioxide was thus produced. Since, moreover, the boiled juices employed invariably contained phosphates, precipitable by magnesia mixture, there can be no doubt that it is to the presence of these that this initial phenomenon is due. Quantitative estimations revealed the somewhat surprising fact that the extra quantity of carbon dioxide evolved in the initial period when a phosphate or a boiled juice is added, corresponds with the evolution of one molecular proportion of carbon dioxide for each atom of phosphorus added in the form of phosphate.

In order to obtain accurate results with solutions of sodium or potassium phosphate, the fact that these absorb carbon dioxide must be taken into consideration. Solutions of the dihydrogen salts of potassium and sodium are too acid to be employed and the monohydrogen salts or a mixture of these with the dihydrogen salts were always used. In every case the liquid before being added to the yeast-juice was saturated with carbon dioxide at the temperature of the bath, and the volume of carbon dioxide liberated by the addition of excess of hydrochloric acid was ascertained in an aliquot portion.

At the close of the fermentation the fermented liquid was acidified and the residual combined carbon dioxide measured, the difference between this and the original amount being subtracted from the amount evolved during the fermentation.

The results are more precise when the yeast-juice employed is an active one, since when the fermenting power of the juice is low the initial period becomes unduly prolonged and the calculation of the extra amount of carbon dioxide is rendered uncertain. The equivalence of the carbon dioxide and phosphate is established by the results contained in the following Table VI. Column 1 gives the observed amount of extra carbon dioxide calculated as described above and reduced to grammes, and Column 2 the equivalent of the phosphate added, this being estimated by precipitation with magnesium citrate mixture in the boiled juice or phosphate solution.

In Experiments 1 to 7 boiled juice was added; in 8 to 14 a solution of sodium or potassium phosphate.

The maximum rate attained during the initial period is from five to eight times as high as the constant rate attained after the evolution of the carbon dioxide equivalent to the phosphate present.

Table VI.—Equivalence of Extra Carbon Dioxide Evolved during the Initial Period, and Phosphate added.

Experiments.	Grammes of carbon dioxide.		Experiments.	Grammes of carbon dioxide.	
	Column I—Observed.	Column II—Calculated from phosphate.		Column I—Observed.	Column II—Calculated from phosphate.
1	0·090	0·086	8	0·196	0·197
2	0·054	0·055	9	0·066	0·065
3	0·058	0·051	10	0·057	0·061
4	0·060	0·049	11	0·056	0·061
5	0·106	0·112	12	0·059	0·061
6	0·103	0·101	13	0·068	0·070
7	0·113	0·112	14	0·071	0·070

At the commencement of the period when sodium or potassium phosphate solution has been added, the rate only gradually acquires its maximum value and sometimes it only attains this maximum after a considerable interval.

This phenomenon is occasionally observed in the fermentation produced by yeast-juice without the addition of phosphate, and also sometimes occurs, but to a much smaller extent, when boiled juice is added. It is well shown in Curve B, fig. 3, which represents the fermentation produced by 25 c.c. yeast-juice + 25 c.c. of a 0·06 molar solution of sodium phosphate + 5 grammes glucose + toluene. The cause of this period of induction has not yet been ascertained.

7. *Limit of the Action of Phosphate.*

If the fermentation in presence of phosphate be allowed to continue until the steady rate is attained and a second quantity of phosphate be then added, a second period of rapid evolution of carbon dioxide sets in and proceeds in a similar manner to the first. This is shown in Curves B and C, fig. 3, which represent the effect of the successive addition of two quantities of 5 c.c. of 0·3 molar sodium phosphate to 25 c.c. yeast-juice + 20 c.c. water, in presence of 10 per cent. glucose. Curve A represents the fermentation in absence of added phosphate. The phosphate solution employed was a mixture of five molecules of NaH_2PO_4 with one molecule of Na_2HPO_4 and no correction for combined carbon dioxide was required. The extra amount of carbon dioxide evolved after each addition is the same, and is equivalent, as already stated, to the phosphate added. The equality is shown graphically in the curve and the equivalence in Experiments 13 and 14, Table VI.

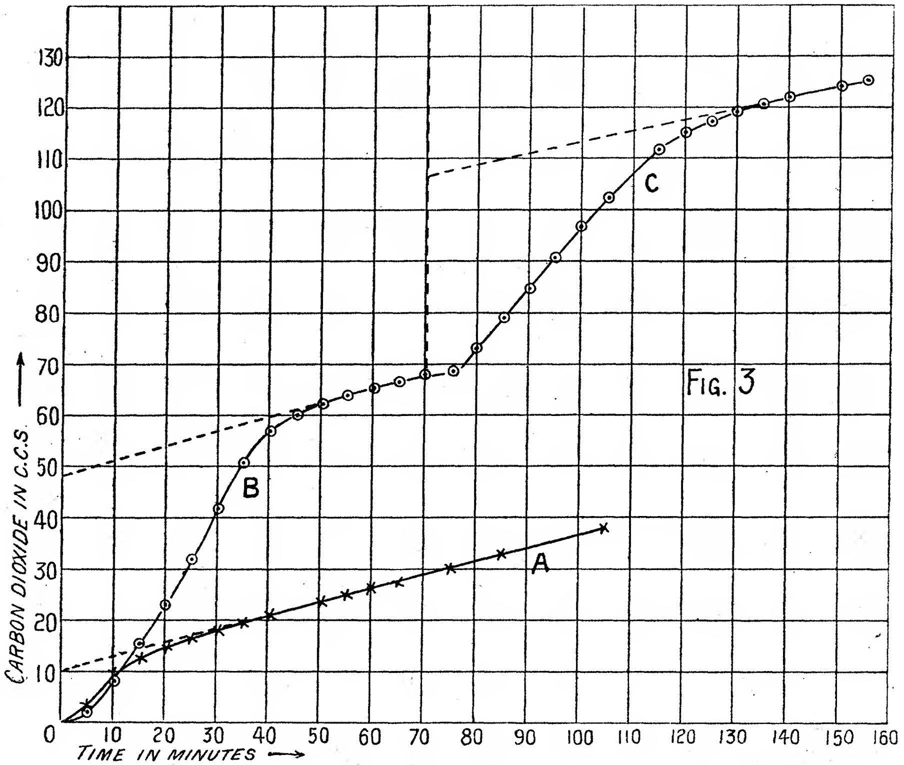


FIG. 3

This process cannot, however, be repeated indefinitely, as after a certain limit is reached the reaction no longer occurs and with a large excess the fermentation is stopped. The exact limit appears to vary both with the nature of the phosphate added and with the particular specimen of yeast-juice employed. The greatest amount of carbon dioxide hitherto obtained in this way from 25 c.c. of yeast-juice is about 0·45 gramme (230 c.c.), which was observed on two occasions, once after the addition of four volumes of boiled juice, and again after the addition of 50 c.c. of a solution of a mixed magnesium potassium phosphate yielding with magnesia mixture 1·187 grammes of magnesium pyrophosphate.

When a specimen of yeast-juice has been incubated until it will no longer ferment sugar, it is not affected by the addition of phosphate.

The fact that the extra carbon dioxide calculated in this way is equivalent to the phosphate present, suggests the superposition of two actions. Whether this is to be explained by the presence of two distinct enzymes or simply by the increased activity of a single enzyme remains to be decided.

8. *Products of Fermentation in the Presence of Phosphate.*

The carbon dioxide evolved during the initial period after the addition of a phosphate is the product of a true alcoholic fermentation of the glucose, in which alcohol and carbon dioxide are produced in equivalent amounts. This was proved in the following way. Twenty-five cubic centimetres of a solution containing 2·5 grammes of glucose and 5 c.c. of a 0·3 molar solution of potassium phosphate were added to 25 c.c. of yeast-juice; the mixture was incubated and the carbon dioxide collected and measured.

As soon as the rate of evolution had become constant, a further addition of 10 c.c. of 0·3 molar phosphate solution was made and the fermentation again continued until the rate had become constant. The gas evolved was tested and found to be carbon dioxide. The total amount evolved during the experiment, which lasted for 2 hours 10 minutes, was 163·4 c.c. at 19°·6 and 758·6 mm. or 0·291 gramme, the equivalent of the phosphate added being 0·196 gramme. The liquid was then distilled with steam and the alcohol estimated in the distillate, 1·312 grammes being found to be present. Twenty-five cubic centimetres of the original juice were found to contain 0·983 gramme of alcohol and therefore $1·312-0·983 = 0·329$ gramme were formed by the fermentation of the sugar. The ratio of alcohol to carbon dioxide produced is therefore $0·329/0·291 = 1·13$, which agrees well with the ratio previously found by similar methods for the fermentation of glucose by yeast-juice.* The theoretical ratio is 1·04.

Lactic acid and acetic acid were also estimated in the original juice and after fermentation in presence of phosphate, but only a very small variation was observed. Twenty-five cubic centimetres of juice gave before fermentation 0·122 gramme of zinc lactate and 0·083 gramme of acetic acid, and after fermentation 0·102 gramme of zinc lactate and 0·072 gramme of acetic acid.

9. *Fate of the Phosphoric Acid.*

When the fermented liquid is boiled and filtered almost the whole of the phosphorus present is found in the filtrate, but it is nearly all in a form which is not precipitated by ammoniacal magnesium citrate mixture.

In the following experiment three quantities of 25 c.c. of yeast-juice were taken:—

A. Hot water was added, the solution heated in a boiling water-bath and the coagulate filtered off and well washed.

B. Ten cubic centimetres of a 30 per cent. glucose solution and 10 c.c. of

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* Harden and Young, 'Ber.,' 1904, vol. 37, p. 1052.

0·3 molar potassium phosphate solution were added and the liquid at once heated to the boiling point, filtered, and the coagulate washed.

C. The same additions were made as to B and the liquid then fermented until the close of the initial period, after which it was heated and filtered like the others.

The total phosphorus was then estimated in each of the coagulates and in each of the filtrates, and the phosphorus precipitated by magnesium citrate in each of the three filtrates. The estimations of total phosphorus were made by heating with sulphuric and nitric acids until colourless, diluting and precipitating with magnesium citrate mixture in presence of excess of ammonia.

The following were the results obtained, the numbers representing the grammes of magnesium pyrophosphate per 25 c.c. of juice.

Table VII.

	A. Original juice.	B. Juice + phosphate. Not fermented.	C. Juice + phosphate. Fermented.
Coagulate	0·053	0·057	0·072
Filtrate—			
(*a*) Precipitated by Mg citrate	0·126	0·480	0·070
(*b*) Not precipitated by Mg citrate	0·271	0·282	0·679
Total	0·450	0·819	0·821

The amount of phosphate added was equivalent to 0·372 gramme of magnesium pyrophosphate.

A number of other results are given to show the extent to which phosphate is converted into the non-precipitable form by this reaction. All the estimations were made by boiling and filtering the fermented liquid immediately upon the close of the initial period. As before the numbers represent grammes of magnesium pyrophosphate obtained from 25 c.c. of juice.

The form in which this non-precipitable phosphorus is actually present in the fermented liquid, and in the liquid which has been boiled and filtered, has not yet been ascertained with certainty. Experiments which are still in progress, however, appear to indicate that it exists in combination with glucose, probably in the form of a phosphoric ester.

Table VIII.—Conversion of Phosphate into the Non-precipitable Form by Yeast-juice and Glucose.

	Phosphate added.	Precipitable phosphate in filtrate.	Non-precipitable phosphate in filtrate.
1	0·553	0·066	1·032
2	0·490	0·090	0·832
3	0·250	0·054	0·685
4	0·488	0·091	1·040
5	0·495	0·088	0·881

The question as to whether the entire phenomenon of the fermentation of glucose by yeast-juice depends on the presence of phosphates has not yet been definitely decided. The addition of phosphate undoubtedly produces a larger increase in the total fermentation than is simply due to the equivalent amount of carbon dioxide evolved in the initial period. The extent of this increase appears to vary very considerably with different specimens of yeast-juice, but the prolongation of the fermentation is not so great as is caused by boiled fresh juice. This question can only be satisfactorily settled by ascertaining whether the addition of a phosphate to the perfectly inactive residue obtained from a juice by filtration through a gelatin filter is sufficient to restore its fermenting power in the same way as the filtrate or a boiled juice. Experiments on this point are in progress, but no decisive result has as yet been obtained, and all discussion of this point will best be deferred until these are completed.

Various other points of interest raised in the course of the investigation, and the study of the relation of these phenomena to the fermentation of glucose by living yeast, are also occupying our attention.

A short outline of the main conclusions arrived at in the foregoing paper, has been previously published in the form of two preliminary communications, without any experimental details.* After the appearance of these notes, Buchner and Antoni† repeated and confirmed a number of the experiments dealing with the effect of boiled juice and of phosphates on the total fermentation, and with the separation of the juice by dialysis into an inactive residue and a dialysate capable of rendering it active. Buchner and Antoni were able, with the more stable juice at their disposal, to carry out the dialysis in the ordinary way for 24 hours and in this manner to confirm the results obtained by the use of the gelatin filter. Owing to the lack of experimental detail, Buchner and Antoni imagined that in our comparative experiments the concentration of glucose and of enzyme had not been kept constant, and ascribed part of the increase produced by boiled juice to the favourable effect of a diminution in the concentration of the sugar and of the alcohol, which is always present, by dilution with the added boiled juice. The details given above show that neither of these influences had any share in the effects observed by us.

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* 'Journ. Physiol.,' 1904, vol. 32; 'Proc.,' of November 12; 'Proc. Chem. Soc.,' 1905, vol. 21, p. 189, June 6.

† 'Zeit. Physiol. Chem.,' 1905, vol. 46, p. 136.

The Quantitative Estimation of Small Quantities of Nickel in Organic Substances.

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In the course of an investigation into the toxic action of certain nickel compounds, it was found necessary to devise a method of detecting and estimating nickel, when included in animal tissue, in quantities not exceeding a few milligrammes per cent.

A method has therefore been worked out, which, although in many respects only differing from the usual methods in virtue of slight alterations of detail, is capable of demonstrating extremely small quantities of nickel accurately. The method may be divided into three stages: (1) The Ashing; (2) The Separating; and (3) The Estimating stages.

1. *Ashing.*—The substance to be examined must be placed in a porcelain crucible (platinum is unsuitable, as a considerable loss of nickel takes place, probably by an alloy of platinum and nickel being formed) and evaporated to dryness over a water bath. If the substance be solid, it should be cut up into small pieces. The crucible is then heated carefully with a Bunsen flame, but it may be wise to further dry in a hot air oven or on a sand bath before this. Then it is burned over a Fletcher burner, and lastly fully incinerated in the blow-pipe flame. With some care, it is possible, as a rule, to oxidise fully all the carbon, without recourse to any foreign material. The crucible is then placed on the water bath, and 10 c.c. of pure hydrochloric acid are added and allowed to evaporate to dryness, this process being repeated. The residue is then extracted with water to which a small quantity of hydro-